Physiological and transcriptomic characterization of a *fliA* mutant of *Pseudomonas putida* KT2440

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Summary

Pseudomonas putida **KT2440 encodes 23 alternative sigma factors. The *fliA* gene, which encodes σ^{28}, is in a cluster with other genes involved in flagella biosynthesis and chemotaxis. Reverse transcriptase-PCR revealed that this cluster is comprised of four independent transcriptional units: *flhAF, fleNfliA, cheYZA* and *cheBmotAB*. We generated a nonpolar *fliA* mutant by homologous recombination and tested its motility, adhesion to biotic and abiotic surfaces, and responses to various stress conditions. The mutant strain was nonmotile and exhibited decreased capacity to bind to corn seeds, although its ability to colonize the rhizosphere of plants was unaffected. The mutant was also affected in binding to abiotic surfaces and its ability to form biofilms decreased by almost threefold. In the *fliA* mutant background expression of 25 genes was affected: two genes were upregulated and 23 genes were downregulated. In addition to a number of motility and chemotaxis genes, the *fliA* gene product is also necessary for the expression of some genes potentially involved in amino acid utilization or stress responses; however, we were unable to assign specific phenotypes linked to these genes since the *fliA* mutant used the same range of amino acids as the parental strain, and was as tolerant as the wild type to stress imposed by heat, antibiotics, NaCl, sodium dodecyl sulfate, H_2O_2 and benzoate. Based on the sequence alignment of promoters recognized by FliA and genome *in silico* analysis, we propose that *P. putida* σ^{28} recognizes a TCAAG-t-N_{12}-GCCGATA consensus sequence located between –34 and –8 and that this sequence is preferentially associated with an AT-rich upstream region.**

Pseudomonas putida KT2440 is a Gram-negative soil bacterium that is able to use a wide variety of organic compounds and efficiently colonize the roots of plants of agronomical interest (Molina *et al.*, 2000). This strain is motile thanks to polar flagella, and it is able to adhere to biotic and abiotic surfaces (Molina-Henares *et al.*, 2006). Two large outer surface adhesion proteins, known as LapA and LapE (Hinsa *et al.*, 2003; Espinosa-Urgel and Ramos, 2004), and type IV pili have been suggested to be involved in attachment to biotic and abiotic surfaces (Hinsa *et al.*, 2003; Espinosa-Urgel and Ramos, 2004). However, the role of flagella in attachment in this strain and other *Pseudomonas* species is controversial (Lugtenberg and Bloemberg, 2004; Yousef-Coronado *et al.*, 2008).

In the genus *Pseudomonas*, as in other bacteria, large numbers of genes are involved in flagellar biosynthesis. In *Pseudomonas aeruginosa* these genes are controlled by a regulatory cascade initiated by the production of the master regulator FleQ and the alternative sigma factor FliA (σ^{28}) (Dasgupta *et al.*, 2003). FleQ, together with RpoN (σ^{54}), induces expression of the class II flagellar genes including *fleSR*, which encode a two-component system that in turn activates class III genes in concert with RpoN. The sigma factor FliA is required for transcription of class IV flagellar genes, among which is *fliC*, which encodes the major flagellin subunit (Dasgupta *et al.*, 2003). This cascade serves to control the timing of gene expression so that it coincides with the assembly of the flagellar apparatus (Prouty *et al.*, 2001; Dasgupta *et al.*, 2003). In Gram-negative bacteria the two flagellar proteins MotA and MotB function together as a complex that generates the torque driving the rotation of the filament (Dasgupta *et al.*, 2004). The flagellar motor of *P. aeruginosa* involves additional Mot proteins, e.g. MotC, MotD and MotY (Doyle *et al.*, 2004; Toutain *et al.*, 2005; Eggenhofer *et al.*, 2006; Yagasaki *et al.*, 2006; Giraud *et al.*,

2009). MotAB together with MotY contribute mainly to motility in liquids, whereas MotC and MotD are less important for swimming (Doyle *et al.*, 2004).

The present study of a *fliA* mutant was undertaken to identify the set of genes under the direct or indirect influence of FliA, to establish whether flagella are important for attachment to surfaces, and to assign new phenotypes linked to *fliA* mutants in *P. putida*.

Inactivation of the *fliA* gene by site-specific mutagenesis

The genome sequence of *P. putida* KT2440 revealed that the *fliA* gene is located within a cluster of genes related to chemotaxis and motility. Located upstream of the *fliA* gene are *flhA*, *flhF* and *fleN*; while the genes *cheY*, *cheZ*, *cheA*, *cheB*, *motA* and *motB* are located downstream (Fig. 1). Before we generated the *fliA* mutant we decided to establish the transcriptional organization of these genes. To this end we carried out a series of RT-PCR assays with RNA isolated from motile *P. putida* cells grown on soft agar using a series of primers based on adjacent genes. Our results revealed four operons in the region, namely, *flhAF*, *fleNfliA*, *cheYZA* and *cheBmotAB* (data not shown). Therefore, *fliA* is the second gene in a bicistronic operon, which, as our results clearly indicated, is not co-transcribed with *cheY*. We subsequently decided to generate a *fliA* mutant using site-directed mutagenesis with the promoterless *aphA-3* gene that encodes kanamycin resistance, and which does not exert any polar effects on downstream genes (Ménard *et al.*, 1993; Duque *et al.*, 2007a; Revelles *et al.*, 2007). To this end a 2.7 kb KT2440 DNA fragment containing the *fliA* gene was amplified with the oligonucleotides 5′-CGAAGC TCATCAAACGTGCT-3′ and 5′-CAAACGTACCCTCGCC GATG-3′ using chromosomal DNA from *P. putida* KT2440 as a template. The amplified fragment was cloned into the pGEM-T vector (Promega) and the resulting plasmid was named pANA177. This plasmid was digested with BsrG1 (which cuts 20 base pairs after the *fliA* start codon), and the overhangs made blunt with dNTPs and Klenow enzyme prior to ligation to a SmaI fragment spanning the *aphA* gene of pUC18K (Parker and Meyer, 2005). The resulting plasmid, pANA178, was then introduced into *P. putida* KT2440 by electroporation and derivatives that had integrated the mutant gene via double homologous recombination were selected on LB solid medium containing 50 μg ml^{-1} kanamycin. From among the set of Km^R clones that we obtained, we searched for those that were unable to grow with 300 μg ml^{-1} piperacillin. A few clones were found, which could have potentially resulted from direct homologous double recombination. We subsequently screened these clones and after PCR and Southern blotting, a mutant designated KT2440 *fliA*::*aphA-3* was chosen for use in further assays. The RT-PCR assays confirmed that the inactivation of *fliA* does not influence the expression of the downstream *cheYZA* genes.

The *P. putida fliA* mutant, when grown on M9 minimal medium with citrate, glucose or benzoate, exhibited growth rates (i.e. doubling time in the range of 50–55 min) and growth yields similar to those of the parental strain, which suggests that no key alterations in basic cell physiology had occurred. We also tested expression of promoters dependent on σ^{70}, σ^{54} and σ^{32}/σ^{38} using transcriptional fusions to *luxAB* or *lacZ* of the σ^{70}-dependent Pr promoter of *dmpR*, the σ^{54}-dependent Po promoter of a (methyl)phenol degradation pathway (Sze *et al.*, 2002) and the σ^{38}/σ^{32}-dependent Pm promoter for the degradation of 3-methylbenzoate (González-Pérez *et al.*, 1999). The pattern of expression of these three promoters was almost identical to that of the parental strain (Ramos *et al.*, 1990; Shingler *et al.*, 1993). In short, Pr was expressed without culture amendments showing maximal activity during the stationary phase (4000 luciferase units). DmpR-dependent Po expression occurred in the presence of 2-methylphenol achieving maximal stationary phase levels of 150 000 luciferase units, whereas Pm expression in a *xylS*$^+$ background occurred in response to 3-methylbenzoate with around a 20-fold induction in β-galactosidase activity. These results suggest that expression from promoters that are not σ^{28}-dependent is unaltered in the *fliA* mutant background.

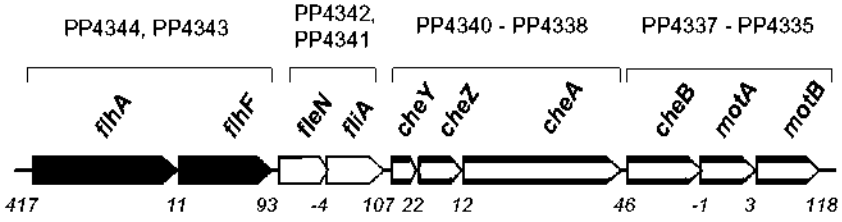

Fig. 1. Physical and transcriptional organization of the *fliA* region of *P. putida*. Gene organization was established by Nelson and colleagues (2002) when the *P. putida* KT2440 genome was determined. The transcriptional organization has been determined in this study (see text). Genes belonging to the same operon are marked with the same shade. The numbers below the arrows represent the intergenic distance between contiguous genes, while negative numbers indicate overlapping genes.

Motility and adhesion properties of the *fliA* mutant

We next determined whether the strain's ability to swim was affected. To this end parental strain and KT2440 *fliA*::*aphA-3* cells were spotted onto 0.3% [wt/vol] LB agar motility plates. We found that the parental strain produced a growth halo of approximately 15 mm in 24 h at 30°C, whereas the *fliA* mutant failed to produce a growth halo. The *fliA* mutant strain was transformed with plasmid pMMB:P_{tac}::*fliA*/*lacI*. In the presence of IPTG the strain produced flagella (V. Singler and S. Österberg, unpublished). We also found that the *fliA* mutant was deficient in adhesion to seeds and that the number of cfu per seed decreased significantly when compared with the parental strain (Fig. 2). The same limited ability of the *fliA* mutant to adhere to biotic surfaces was also observed for abiotic surfaces (i.e. polypropylene, borosilicate and polystyrene surfaces), resulting in limited biofilm formation, which was in mass terms quantified as 1/2 to 1/3 that of the parental strain (Fig. 3).

Electron microscopy analysis revealed that KT2440 has six or more polar flagella (Fig. S1), and to ascertain whether the limited ability of the *fliA* mutant to form biofilms was due to the lack of flagella, we chose to assess whether the phenotype of the *fliA* mutant was similar to *fliC*, a mutant that was unable to produce flagella. The *fliC* mutant exhibited a phenotype that was similar to the *fliA* mutant – assays showed that it was nonmotile and that it adhered to surfaces in a less efficient manner than the parental strain; i.e. adhesion to corn seeds was fivefold less efficient, and its ability to form biofilm in mass terms was about 1/3 of that of the parental strain (not shown). Therefore, our results provide evidence that supports that KT2440 flagella are involved in adhesion to surfaces.

Next we tested whether deficiency in attachment of the *fliA* and *fliC* mutants had an effect on root colonization in assays in which individual strains or combinations of KT2440/*fliA* or KT2440/*fliC* mutants were used to inoculate corn seeds (Espinosa-Urgel and Ramos, 2004). Colonization rates by *fliA* and *fliC* mutants were around 2.7 ± 0.3×10^7 cfu g root^{-1}, which were similar to rates found for the parental strain ($3 \pm 0.2 \times 10^7$ cfu g root^{-1}). In corn root co-inoculation assays of the wild type and one of the mutants, we found that mutant strains reached cell densities similar to those found for the wild-type parent strain. The analysis of two independent events – attachment and colonization – suggest a role of flagella in surface binding. Proliferation in the root system seemed unaltered, and may be mediated through a swarming-like movement described recently for this strain (Matilla *et al.*, 2007).

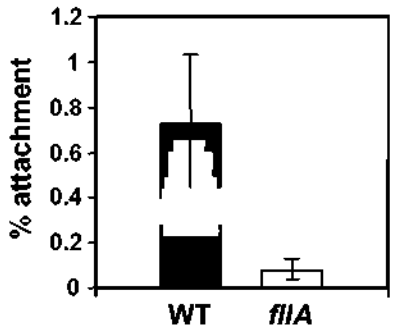

Fig. 2. Adhesion of *P. putida* KT2440 [wild type (wt)] and the FliA mutant to corn seeds. Quantification of attachment of *P. putida* KT2440 and mutant FliA to corn seeds was as described by Espinosa-Urgel and Ramos (2004). After 1 h of incubation in each bacterial suspension, seeds were washed and disrupted, and the number of attached cells was estimated as cfu after plating serial dilutions. The results are presented as percentage of attached cells (average ± standard deviation) with respect to the number of inoculated cells (average of at least four independent experiments).

Array assays

To identify the set of genes under the influence of FliA, parental KT2440 and the *fliA*::*aphA-3* mutant cells were grown on LB and mRNA was prepared from cells growing exponentially on LB liquid medium for transcriptomic analysis as described before. The genome-wide DNA chip used in this work was printed by Progenika Biopharma

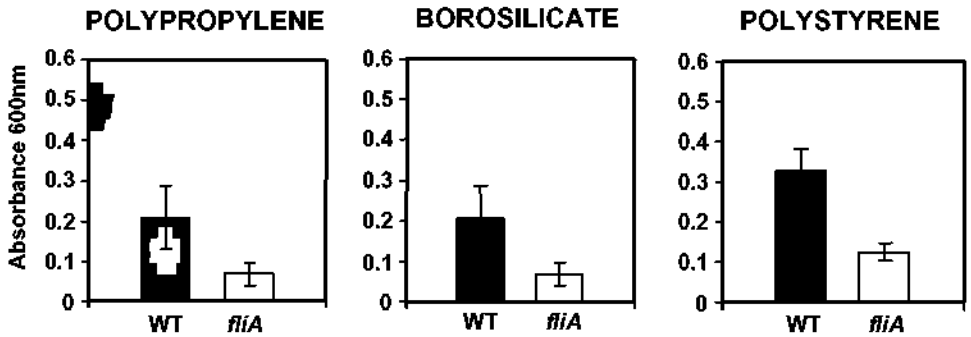

Fig. 3. Biofilm formation assay. Biofilm formation on different abiotic surfaces, quantified by staining attached cells with the crystal violet method (Hinsa *et al.*, 2003). Ethanol-solubilized crystal violet was measured at A_{600}. The results are the average of three independent assays.

(Yuste *et al.*, 2006; Duque *et al.*, 2007a,b). Preparation and labelling of RNA for hybridization was done as previously described (Yuste *et al.*, 2006; Duque *et al.*, 2007b).

The number of genes under the direct or indirect control of *fliA* was found to be limited to 25, of which two (PP3099 and PP3100) were upregulated; the function of these proteins is unknown. The rest of the genes were downregulated. As expected, most of the downregulated genes were related to motility functions (see Table 1). Levels of the *fliC* and *flaG* genes showed a more than fivefold decrease, and genes encoding chemotaxis proteins such as CheB, CheY, the Aer-2 aerotaxis protein and the methyl-accepting chemotaxis transducer proteins PP0584, PP1371 and PP2249 were also among the downregulated genes. We analysed in detail the array data regarding all *fli, che, mot, fle, flg* genes because they encode functions related to flagellar biosynthesis or motility. Apart from the genes shown in Table 1 the level of expression of the rest of these genes was not significantly different to that of the KT2440 parental strain in the *fliA* mutant (fold changes were in the range between −1.56 and 1.48).

A surprising finding was the decreased expression of three regulatory proteins, namely PP4400, PP0914 and PP5324. The BkdR (PP4400) regulator is involved in the biosynthesis of enzymes for branched amino acid metabolism; PP0914, with a GGDEF-EAL domain may be involved in regulatory cyclic-di-GMP signalling, while no function can be envisaged for the putative PP5324 response regulator. One possible hypothesis is that these three regulatory proteins could be responsible for the repressed expression of other genes. For example, we noted a decrease in the expression of *orfPP4403*, which encodes the lipoamide acyltransferase of 2-oxoisovalerate dehydrogenase BkdB involved in branched amino acid metabolism, and which may therefore be under the control of the BkdR regulator.

The *fliA* mutant exhibited decreased levels of expression of genes that encode enzymes related to amino acid metabolism such as asparaginase and an isochorismate hydrolase (Table 1). We also found two genes that encode proteins of unknown function to be downregulated, i.e. PP3642 and PP4448. The possibility that FliA might control genes other than those related to flagella biosynthesis has also been reported in the enteric bacteria *Xenorhabdus nematophila* in which FliA controls biosynthesis of the XlpA lipase and the XrtA protease (Park and Forst, 2006). In other bacteria, σ^{28} recognizes the promoters of genes involved in several functions, including sporulation and the response to certain forms of stress (Josenhans *et al.*, 2002). Based on our microarray data and the set of findings in other microorganisms, we decided to compare some of the physiological traits of the *P. putida fliA* mutant and its wild-type parental

Table 1. Genes downregulated (fold change < −1.8) in *P. putida* KT2440 *fliA* (versus *P. putida* KT2440).

TIGR identifier	Gene product	Gene name	Change (fold)	Role cat.[a]	Putative FliA box[b]
PP_0076	Glycine betaine-binding protein, putative		−2.1	3	
PP_0584	Methyl-accepting chemotaxis transducer		−2.0	1	
PP_0779	Methyl-accepting chemotaxis transducer/sensory box protein		−2.1	1	Y
PP_0913	Conserved hypothetical protein		−1.8	5	
PP_0914	GGDEF domain protein		−2.0	5	Y
PP_1160	Asparaginase family protein		−1.9	2	
PP_1371	Methyl-accepting chemotaxis transducer		−2.7	1	Y
PP_1467	Sodium/hydrogen exchanger family protein		−2.4	3	
PP_2111	Aerotaxis receptor Aer-2	*aer-2*	−2.1	1	Y
PP_2249	Methyl-accepting chemotaxis transducer		−2.1	1	Y
PP_3642	Hypothetical protein		−2.6	5	
PP_4337	Protein-glutamate methylesterase CheB	*cheB*	−2.1	1	
PP_4340	Chemotaxis protein CheY	*cheY*	−2.8	1	
PP_4375	Flagellar biosynthesis protein FliS	*fliS*	−1.9	1	Y
PP_4376	Flagellar cap protein FliD	*fliD*	−2.9	1	
PP_4377	Flagellin FlaG, putative	*flaG*	−5.1	1	
PP_4378	Flagellin FliC	*fliC*	−6.5	1	Y
PP_4395	Negative regulator of flagellin synthesis FlgM	*flgM*	−1.8	1	Y
PP_4400	Transcriptional regulator BkdR	*bkdR*	−2.4	4	
PP_4403	2-oxoisovalerate DHase, lipoamide acyltransferase component	*bkdB*	−4.7	2	
PP_4448	Conserved hypothetical protein		−1.8	5	
PP_5243	Hydrolase, isochorismatase family		−1.9	5	
PP_5324	Putative response regulator		−2.8	4	

a. Role categories: 1, chemotaxis and motility; 2, energy metabolism (amino acids and amines); 3, transport and binding proteins; 4, regulatory functions; 5, hypothetical proteins and proteins of unknown function.

b. Presence of a putative FliA recognition motif within the 300 bp region immediately upstream of the annotated translational start codon of the corresponding gene: Y = yes (motif found by the BioProspector program using the parameters defined in the text). A blank space indicates that no putative FliA-like binding motif was found for that gene.

strain under different growth conditions, i.e. growth in the presence of high NaCl concentrations, heat shock tolerance, tolerance to heavy metals, metalloids, paraquat, H_2O_2, sodium dodecyl sulfate, toluene and benzoate. We also tested utilization of branched amino acids and other amino acids as a nitrogen source. Both the parental strain and the *fliA* mutant grew in ELISA plates with shaking in citrate M9 minimal medium with 1.2–1.4 M NaCl, 5% (w/v) sodium dodecyl sulfate, 5 mM H_2O_2, 1 mM paraquat, a 1% (w/v) toluene and 25 mM benzoate. Both the *fliA* mutant and the wild-type strain grew in the presence of up to 20 mM $MnCl_2$, 20 mM $CuCl_2$, 0.66 nM $NiCl_2$ and > 1 mg l^{-1} arsenite. Both the parental and *fliA* mutant strains exhibited similar death kinetics when culture temperatures were increased from 30°C to 45°C (not shown).

Because some of the downregulated genes in the *fliA* mutant might be involved in the catabolism of valine (PP4403) and asparagine (PP1160), we tested growth of the *fliA* mutant on minimal medium with 10 mM citrate and the above amino acids as N-sources supplied at 5 mM. The FliA mutant could use both amino acids with doubling times similar to those of the parental strain.

Stevenson and Rather (2006) found that decarboxylated amino acids inhibit the flagellar regulon of *Proteus mirabilis*, although the molecular basis of the phenomenon is unknown. Work at our laboratory showed no changes in the pattern of expression of the flagellar genes in *P. putida* growing in the presence of phenylalanine and tyrosine (C. Herrera and J.J. Rodríguez-Herva, unpublished), but whether or not the metabolism of other amino acids actually influences expression of σ^{28}-dependent genes in *P. putida* is unknown. Microarray assays with *Escherichia coli* have shown that flagellar synthesis and motility were repressed at high pH (Maurer *et al.*, 2005); however, no genes related to pH homeostasis were up- or downregulated in *P. putida* KT2440, which suggest that this type of control may not be universal or that it is not mediated by FliA in KT2440. Therefore, our results indicate that the most clear phenotype of the *fliA* mutant *P. putida* KT2440 is the lack of motility and the decreased ability to adhere to biotic and abiotic surfaces.

Physical and transcriptional organization of the FliA-controlled genes as a clue to the identity of *P. putida* FliA-dependent promoters

FliA, like other σ^{70} family members, confers sequence specificity to core RNA polymerase with the conserved 2.4 and 4.2 regions being intimately involved in recognition of the −10 region and −35 regions of their cognate promoters (Kuznedelov *et al.*, 2002; Koo and colleagues 2009). As expected, alignment of the primary sequence of *P. putida* FliA with other FliA proteins including the *E. coli* σ^{28} protein revealed high identity along the entire sequence with homology particularly well conserved in the segments corresponding to regions 2.4 and 4.2 of σ^{70}-family proteins (Fig. S2). The above transcriptomic analysis showed that most of the genes under the direct or indirect control of *fliA* are single transcriptional units; however, eight genes are encompassed in three potential operons, i.e. PP0913 with PP0914, PP4337 through to PP4335 and PP4340 to PP4338. These last two groups of genes encode proteins related to motility. We were therefore left with 18 potentially downregulated promoters. To identify a potential consensus motif recognized by the *P. putida* FliA protein, we first considered the consensus sequence derived from FliA-regulated genes of *E. coli*. The *E. coli* FliA consensus sequence, 5′-TAAAGTTT-N_{11}-GCCGATAA-3′, lies between −34 to −8 and has been derived from the alignment of a set of 12 promoters (*aer*, *flgK*, *flgM*, *fliC*, *fliD*, *flxA*, *motA*, *tar*, *trg*, *tsr*, *ycgR*, *yhjH*) (Chilcott and Hughes, 2000; Serizawa *et al.*, 2004; Shen *et al.*, 2006; Koo *et al.*, 2009). Within this sequence the best conserved element is TAAA-N_{15}-GCCGATA. Mutational studies in *E. coli* have shown the critical role of the TAA sequence in the −35 region and the extended GCCG sequence in the −10 region (Yu *et al.*, 2006a,b; Zhao *et al.*, 2007; Wozniak and Hughes, 2008; Koo and colleagues, 2009). We scanned the regions upstream from the putative *fliA*-controlled genes of *P. putida* KT2440 and found sequences similar to the 5′-TAAA-N_{15}-GCCCATA-3′ sequence present in eight of the potential promoters (Fig. 4). We then aligned the upstream region of these promoters from the putative +1 to −50 and found that the best consensus for *P. putida fliA* was TCAAG-t-$N_{12/13}$-GCCGATA, a sequence found upstream of the ORF for PP0779, PP0914, PP1371, PP2211, PP2249, PP4375, PP4378 and PP4395. We propose that this set of genes is transcribed by RNA polymerase with FliA as the sigma factor in *P. putida*. The other set of genes whose level decreased in the absence of FliA may be regulated as a consequence of the decreased expression of regulatory proteins mentioned previously, one of the most obvious cases being the *bkdB* gene, whose expression decreased by fourfold concurring with the decrease in expression of its cognate regulator BdkR.

In silico analysis of chlamydial FliA-dependent promoters suggested that the level of σ^{28}-directed transcription is positively associated with AT-rich sequences upstream from the −35 element in FliA-dependent promoters (Yu *et al.*, 2006a,b). We therefore searched for A + T richness within the −35 to −60 region of the eight FliA-regulated promoters in *P. putida* KT2440. We found that the FliA-dependent promoters generally have high A + T contents (56–76% A + T; Fig. 4), particularly considering that the

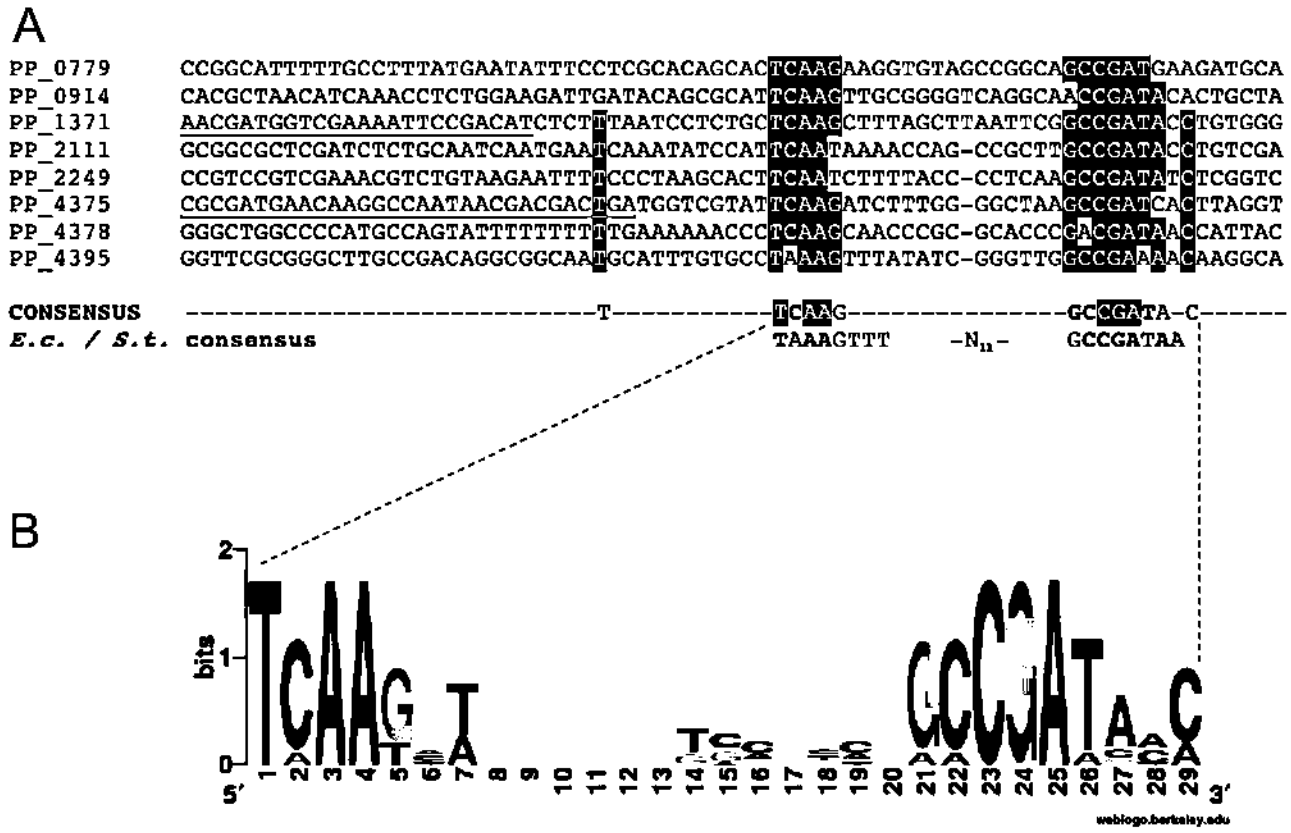

Fig. 4. Analysis of the *P. putida* KT2440 FliA DNA binding motif.
A. Multiple alignment of the DNA regions upstream of the translational start codon of eight genes selected by the BioProspector program (among all of the downregulated genes found in *P. putida* KT2440 *fliA*) as potentially containing a FliA binding motif. Underlined nucleotides belong to the coding region of the upstream adjacent gene. Nucleotides present at the same position in at least six of the eight sequences (shaded in black in the alignment) were included in the consensus sequence. Black-shaded nucleotides (in the consensus sequence) indicate positions conserved in all eight sequences. Nucleotides present in seven out of eight sequences are highlighted in bold. For comparison purposes, the consensus binding motif of the *E. coli* (E.c.) and *Salmonella enterica* sv. typhimurium (St) sigma F protein is shown on the bottom line (with most conserved positions displayed in bold).
B. Frequency diagram (logo) of the consensus sequence derived from the predicted *P. putida* KT2440 FliA DNA binding motif. The relative height of the letters is proportional to their frequency at a particular position. The logo was generated by Weblogo (Crooks *et al.*, 2004).

overall A + T content of the *P. putida* genome is 38.48% and that in the intergenic regions it reaches 44.43%. However, no clear consensus sequence was found when this region was analysed.

To further assess whether A + T richness in the region between –35 and –60 influences transcriptional activation of FliA-dependent promoters, we decided to generate a chimeric promoter that contains the –35/–60 region of a non-FliA-regulated promoter with a low A + T content (~40% A + T) fused to the –34/+1 region of a FliA-dependent promoter. For this, we chose PP1371, a gene whose expression is FliA-dependent, to provide the +1/–34 region, while for the –35/–60 region we chose the non-FliA-dependent gene encoding PP2128, which has a A + T content of 32%. We then specifically synthesized a 60-mer double-stranded oligonucleotide corresponding to the chimeric promoter and cloned it upstream from *'lacZ* in pMP220 (Spaink *et al.*, 1987). As a control we synthesized a double-stranded 60-mer oligonucleotide that corresponded to the entire sequence of PP1371 and also cloned it upstream of *'lacZ*. β-Galactosidase activity was determined in KT2440 at the end of the log phase (OD_{660} around 2). We found that the incorporation of the A + T-poor region of PP2128 upstream of PP1371 resulted in a drop in activity (1200 units for the wild-type promoter versus 300 units for the chimeric promoters). This suggests that the A + T-rich region upstream of –35 functions as an enhancer-like UP element that influences promoter expression. Indeed, the chimeric promoter in which the –35/–60 region of PP2128 was replaced by that of PP1371 yielded a promoter whose activity was twice that of the PP2128 promoter (not shown).

To conclude, *P. putida* KT2440 deficient in FliA is non-motile and its adherence to biotic and abiotic surfaces is compromised. We have identified a specific set of eight promoters that are transcribed by RNA polymerase utiliz-

ing FliA (σ^{28}), and shown that these promoters exhibit a conserved sequence between positions −34 and −8, defined by the 5′-TCAAG-t-N_{12}-GCCGATA-3′ consensus sequence. Our results also imply that an AT-rich upstream region is associated with these promoters and that it may function as a potential UP-element.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Detail of polar flagella of *P. putida* KT2440 (× 200 000). The picture is courtesy of Dr Sun Nyunt Wai (Umea University, Sweden) and Akemi Takade (Nyushu University, Japan).
Fig. S2. FliA alignment. Multiple alignment of the deduced amino acid sequence of the FliA proteins of *Pseudomonas putida* (Pp), *Pseudomonas aeruginosa* (Pa), *Escherichia coli* (Ec) and *Salmonella enterica* sv. typhimurium (St). Amino acids present in at least two of the four positions were included in the consensus sequence (cons). Residues conserved are shaded in black. The putative 2.4 and 4.2 sigma domains are boxed.
Table S1. Genome-wide computational search for the consensus sequence TCAAG-N14/15-GCCGATA in the upstream intergenic regions of *P. putida* KT2440 genes.